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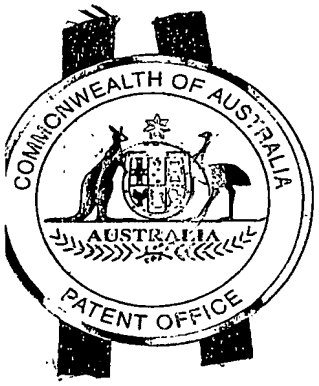

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AUSTRALIA

Patents Act 1990

PROVISIONAL SPECIFICATION

Invention Title: **Formulation method**

The invention is described in the following statement:

FORMULATION METHOD

The present invention relates to methods for the formulation of products using dense gases. It has particular but not exclusive application to the precipitation and encapsulation of particulate products.

Particulate products are of great interest for pharmaceutical applications, where there is a need to obtain particles of reproducible, preferably small, size and shape. These physical criteria are important because the bioavailability of some pharmaceuticals is dependant on the size of the particles. Similarly, bioavailability may be adjusted by coatings (ie, encapsulation) or dispersion (eg, in a polymer matrix, particularly biodegradable polymers).

There are a number of dense gas techniques commonly used in the micronisation of particles. The two techniques particularly relevant to the present invention are Rapid Expansion of Supercritical Solutions (RESS) and Particles from Gas Saturated Solutions (PGSS).

The RESS process involves the material of interest being dissolved in a supercritical fluid solvent under pressure, and precipitating the solute by depressurising the solution across a nozzle.

The PGSS process involves applying a dense gas under pressure to a molten material. The dense gas dissolves in the material of interest to form a solute saturated solution, and the resulting liquid phase is sprayed through a nozzle into a vessel of lower pressure, which results in the dense gas being vaporised, leaving behind fine particles of the desired material. A typical apparatus for this process is illustrated schematically in Figure 1 and described in more detail below.

One known formulation method (which may be used, for example, for delayed release) is to spray a molten pharmaceutical (or material of interest) into a solution of a sustained release compound (such as stearate) at increased temperature and pressure. This results in the newly formed particles of the

pharmaceutical being coated in the stearate (or other similar compound) for delayed release or other applications. The utility of this method for pharmaceutical applications is restricted to the few pharmaceutical substances known to melt without decomposing.

Co-formulation of pharmaceuticals have also been proposed for increased efficacy or new applications. These may however be difficult to prepare, particularly if melting a compound so as to mix it with another partly decomposes it.

In attempting to overcome some of those difficulties and limitations, it has surprisingly been found that some compounds exhibit a melting point depression when exposed to a dense gas, which permits use of PGSS with such compounds which might otherwise have been predicted to be unsuitable given their melting point at atmospheric pressure.

Summary of one form of the invention

This invention is directed to substances whose melting point is depressed in the presence of a dense gas.

In one embodiment, there is provided a method for manipulating or formulating a substance comprising heating the substance in the presence of a dense gas such that the substance melts at a lower temperature than it would under atmospheric pressure. This result is achieved despite the higher pressure experienced by the substance given the presence of the dense gas, which would normally be expected to increase the melting point.

Preferably, the method is conducted in a sealed chamber containing the dense gas.

Preferably, the dense gas is CO_2. Preferably, the substance is a pharmaceutical or biological compound. Examples include cyclosporin and ibuprofen. Usually the substance will be solid at atmospheric pressure and temperature.

The invention has particular advantage where the substance undergoes degradation or decomposition at temperatures between its lowered melting point and up to about its melting point at atmospheric pressure. This is because the substance can be melted with reduced degradation or decomposition

The invention can also be used to encapsulate a material, such as a pharmaceutical substance. In one particular application, where it is desirable to release one drug before another into a metabolic system, the latter drug may be coated in the former by means of this process. This would be particularly suitable if, for example, the first drug aided absorption of the second, which was the primarily active drug. One proposed example of this application of the invention is paclitaxel coated with cyclosporin. Combinations of immunosuppressives are also contemplated. Accordingly, in one application of the invention, paclitaxel is coated with molten cyclosporin at temperatures only moderately above atmospheric.

In another embodiment, this method can be used to produce micronised particles of a material which are encapsulated by a polymer. This polymer coating can be selected to impart various properties to the particles, for example, to enable sustained or delayed release of the encapsulated material. For example, the molten substance under the dense gas could be depressurised through a nozzle so as to precipitate fine particles of the substance. These can then be coated with known coatings (eg, stearate or polylactides) to formulate the substance for medical administration. The invention can also enable a compound, particularly a lipophillic compound, to be embedded into a lecithin vesicle by depressurising into an aqueous solution to produce an emulsion.

In addition, the invention may be used to facilitate administration of pharmaceuticals which are themselves difficult to administer, such as pharmaceuticals having low blood solubility. In place of known techniques whereby micro-emulsions of such pharmaceuticals may be formulated for administration to patients, the invention can be used to coat nano-sized particles of the active ingredient in a compound which facilitates blood solubility and is, itself, biodegradable.

The invention may also be used to formulate micron-sized or nano-sized particles of thermally labile compounds as these can be manufactured using the invention well below the decomposition temperature of the pharmaceutically active substance itself yet still be formed into very small particles. The invention also avoids the polymorphism of crystal structure which often results from known methods (eg, crystallising particles from ethanol). Polymorphism can significantly change the bioavailability of a substance, which in turn may require new regulatory approval. Thus, the ability to formulate a substance by melting it at significantly lower temperatures is significant to avoid decomposition, and the rapid formation of the particles (with greater control over the system compared with known techniques) reducing the likelihood of polymorphic forms of the substance being generated.

The invention may also be used to separate optical isomers where one isomer has a different melting point in the presence of dense gas, by separating the liquid phase (having one isomer) and the solid phase (having the other isomer) of a racemic mixture of isomers. The differential in the isomers' melting points may arise from different temperatures or different pressures of dense gas.

Advantages of the present method include:

(i) the method can be conducted without the presence of organic solvents;

(ii) since the materials melt at a lower temperature than normal, the method is suitable for thermally labile compounds and core or coated compounds;

(iii) the method is more energy efficient than some other dense gas processes, because lower temperatures and/or pressures are used;

(iv) the core drug is protected;

(v) less dense gas is needed, which saves costs.

In the description, the term "dense gas" means a fluid substantially near or above its critical pressure (Pc) and temperature (Tc). In practice, the pressure of the fluid is likely to be in the range (0.5-1.5)Pc and its temperature (0.5-1.2)Tc. The terms "dense fluid" and "expanded fluid" are used synonymously in this specification.

It will be understood that the term "comprises" (or its grammatical variants) as used in this specification is equivalent to the term "includes" and should not be taken as excluding the presence of other elements of features.

Without being bound by any particular theory or mode of action, it appears that this melting point depression is caused by the adsorption of the dense gas into the solid matrix and the resulting solute-solvent intermolecular interactions. The dense gas therefore effectively dissolves in the liquefied Cyclosporin A.

Brief Description of the Drawings

Figure 1 shows schematically the apparatus used to perform the method of the invention.

Figure 2 shows a pressure-temperature diagram for the Cyclosporin A-CO_2 system.

Figure 3 is a diagram showing the solubility of Cyclosporin A at various pressures.

Figure 4 shows a Scanning Electron Micrograph (SEM) image of a sample of Cyclosporin A; (a) before processing using the method of the present invention; (b) after being processed using the method of this invention at 25°C and 160 bar with a 10mm long, 50 micron diameter nozzle.

Figure 5 shows an SEM image of cyclosporin A processed according to the invention at 45°C and 200 bar with a 10mm long, 50 micron diameter nozzle.

Figure 6 shows an SEM image of cyclosporin A processed according to the invention at 25°C and 200 bar with a 10mm long, 100 micron diameter nozzle.

Figure 7 shows SEM images of cyclosporin A (a) before process, (b) after processed by RESS.

Figure 8 shows XRD analysis for cyclosporin A.

Figure 9 shows DSC analysis of cyclosporin A, unprocessed and processed by RESS.

Figure 10 shows particle size distribution of cyclosporin A, (a) unprocessed powder, (b) processed by RESS.

Examples

Example 1

Cyclosporin A, one of the cyclosporin family, is an immunosuppressant used, for example, to prevent organ rejection in transplant patients, and has a melting point of 120-190°C, depending on its crystalline structure (its white prismatic needles have an mp of 148-151°). However, Cyclosporin A in its solid form melted when exposed to carbon dioxide at 45°C and 190 bar.

A phase behaviour study was conducted to determine the optimum conditions for the melting point depression, the solubilisation of Cyclosporin A in CO_2, and for the particle formation. Figure 2 shows the melting point depression of Cyclosporin A at various pressures by a pressure-temperature diagram for the Cyclosporin A-CO_2 system. Figure 3 shows the solubility of Cyclosporin A at various pressures. The solubility of Cyclosporin A in dense gas CO_2 is significant, and its solubility increased as the pressure of the system was changed from 100 to 180 bar.

The phase observation study of the solute-CO_2 system was carried out

using a static technique. A glass tube (i.d. = 5.8 mm) loaded with the solute cyclosporin A was placed inside the view cell (Jerguson sight gauge series No. 32). The system was then immersed in the constant temperature water bath. Prior to commencing experiments, the system was purged with low pressure CO_2 in order to remove moisture and air. Carbon dioxide was gradually fed into the view cell at 3 bar increments. The system was isolated and equilibrated for at least 10 minutes after each increase in pressure in order to observe any phase transition of the solute.

The melting point of cyclosporin A was depressed when contacted with CO_2 at 45°C and 190 bar. The normal melting point of cyclosporin A is a function of its crystal structure and varies between 120°C and 190°C. The pressure temperature diagram for the cyclosporin A-CO_2 system is presented in Figure 2. As the data in Figure 2 shows, upon increasing the CO_2 pressure, the temperature at which cyclosporin A melted increased, but the melting point was still well below the usual melting point.

The behaviour was also observed in ibuprofen-CO_2 systems. The normal melting point of ibuprofen is below 80°C which is much closer to the critical point of CO_2 compared with cyclosporin A. The trend that was observed in cyclosporin A-CO_2 is common for systems where the solid is a heavy and low volatile compound with a critical point far from the critical point of the dense gas.

The solubility of cyclosporin A in dense gas CO_2 was significant. The solubility of cyclosporin A was increased as the pressure of the system was changed from 100 to 180 bar (Figure 3). The degree of solubility was slightly increased when the temperature increased from subcritical (25°C) to supercritical (45°C) conditions. Thus, it can be seen that, due to the considerable solubility of cyclosporin A in CO_2 and melting point depression behaviour, the RESS and PGSS processes can be the efficient methods for micronisation of this compound.

Example 2

The schematic diagram of the PGSS apparatus that was used for the micronisation of Cyclosporin A is shown in Figure 1. The high pressure cell 1 was loaded with a known amount of the drug and then placed in the water bath 2, heated by thermostat heater 3, to keep the temperature constant. Carbon dioxide was then gradually added to the high-pressure cell via the syringe pump 4 and valves 5 and 6, to reach the operating pressure. The system was left isolated in the water bath for at least two hours before spraying to approach equilibrium. The spraying was performed with the pump running at constant pressure mode. The nozzle 7 (50 and 100 μm) was used for spraying the gas saturated solution into the expansion chamber 8. The powder was then collected for characterisation. The gas then continues through a filter 9 which is between the expansion chamber 8 and the scrubber 10, through the scrubber and then exits through the vent 11.

The original Cyclosporin A contained irregular crystalline powder with particles in the range of 50 μm (Figure 4(a)). The particles produced by the PGSS process at 25°C and 160 bar were on average 5 μm microsphere particles (Figure 4(b)).

Example 3

The parameters of the melting point depression observed for cyclosporin A were then analysed as follows. The melting point of the drug decreased from 120°C to 25°C, 35°C, 40°C and 50°C when pressurised with CO_2 at 53, 58, 60 and 77 bar, respectively. Micronisation of the cyclosporin A by the Precipitation from Gas Saturated Solution (PGSS) process is thus efficient due to the significant drop in melting point at relatively moderate pressures.

The effect of temperature (between 25°C and 45°C), pressure (between 100 and 200 bar) and nozzle diameter (50 and 100 μm) on the characteristics of the precipitates and the PGSS performance was examined. As shown in Figure 5, the particle size of cyclosporin was not significantly influenced by the pressure and temperature of the system (cf figure 4(b) – particles produced at 25°C and 160

bar. However, the PGSS process was more efficient at high pressures, such as 200 bar, and temperatures such as 45°C. At high pressure the solubility of CO_2 in the liquid phase (melted cyclosporin) was increased, which lead to a decrease in the liquid viscosity. The viscosity of the liquid phase can also be decreased by raising the temperature of the process. Both factors can improve the mobility of the liquid phase, minimise the nozzle blockage and make the PGSS process more practical.

The characteristics of the powder produced by PGSS process is also significantly influenced by nozzle dimension. The nozzle geometry influences the fluid flow characteristics, pressure drop along the expansion pathway and the atomization of the solution. Increasing the nozzle diameter may result in generation of larger particles due to a decrease in pressure drop and density of the fluid at the exit of the nozzle.

In this study it was found that the nozzle dimension possessed a significant effect on the particle size of cyclosporin processed by PGSS. As can be seen in Figure 6, the particles produced from a 100 μm nozzle were porous and significantly larger than the ones precipitated from a 50 μm nozzle (see figures 4(b) and 5). Without wishing to be bound by any specific modality of operation, it is believed that the porous structure that was observed in the microspheres might be caused by diffusion of carbon dioxide from the microspheres during the expansion stage.

Example 4

Drugs which may be used in combination with cyclosporin include Basiliximab, Tacrolimus, Docetaxel. There is evidence (ref: *J Clin Oncol* 2001 Feb 15; 19(4): 1160-6) that the bioavailability of docetaxel is strongly enhanced by coadministration of cyclosporin A. Thus, the invention enables the encapsulation of a compound such as these with cyclosporin.

In an alternative embodiment, cyclosporin A may itself be coated, such as by polycaprolactone into nanoparticles. This has been shown to improve the oral

bioavailability of cyclosporin A and its uptake by lymphocytes, without a corresponding increase in immunosuppression and adverse effects.

Cyclosporin A can also be incorporated into lecithin vesicles, as cyclosporin is lipophillic. It is first melted under dense gas and then mixed with a phospholipid, such as lecithin. A surfactant, preferably a non-ionic surfactant (eg, polysorbate, TWEENs, SPANs, polyethoxylated castor oil, etc) may also be added at this point. The mixture is then depressurised into an aqueous solution (rather than into air as in the previous examples). The resulting solution will be an emulsion containing cyclosporin in small vesicles or micelles. This is an efficient way of generating a cyclosporin (or other lipophillic compound) aqueous emulsion, without the cyclosporin decomposing.

Improved bioavailability of cyclosporin A has also been shown by forming microspheres containing cyclosporin A and sodium lauryl sulphate ("SLS"). In particular, cyclosporin A, SLS and dextrin in the ratio of 1:3:1 has been found very effective. The invention can utilise the decreased melting point of cyclosporin A in the presence of dense CO_2 to create such spheres by then mixing it with the SLS and dextrin in the required ratios.

The invention is equally applicable to cyclosporin derivatives, such as valspodar.

Example 5

Particles were produced by a RESS process to enable a comparison to be made between particles produced using the RESS and PGSS process.

The original material of cyclosporin A contained irregular particles, 100 μm average particle size and with a broad particle size distribution. The product produced from cyclosporin A – CO_2 system by RESS at both 25°C and 40°C and 180 bar were uniform microsphere particles. The particle size was substantially reduced to about 200 nm as depicted in Figure 7.

In summary, cyclosporin A can be micronised by both RESS and PGSS processes. It is also possible to produce porous structure particles that may improve the dissolution rate of the poorly water soluble cyclosporin. The particles formed by the RESS process were smaller, with narrower particle size distribution than the product produced from the PGSS technique. However the yield of the process was higher in the PGSS than in the RESS process.

The degree of crystallinity and polymorphic form of the cyclosporin was examined by x-ray diffraction (XRD) and differential scanning calorimetry (DSC). The results obtained from XRD analysis shown in Figure 8 indicate that the original powder was in crystalline form. The DSC results presented in Figure 9 indicate that the unprocessed crystal of cyclosporin A has the melting point of 120°C. Both XRD and DSC confirmed that the cyclosporin powder processed by RESS has no peak at regions that crystal forms of the cyclosporin exist, hence the product is in amorphous form.

The particle size distribution of the cyclosporin A powder was measured by laser diffraction (Master Size, Malvern Instruments, UK). As demonstrated in Figure 10, which shows the particle size distribution of cyclosporin A (a) unprocessed, and (b) processed by RESS, the average particle size and particle size distribution of the cyclosporin processed by RESS was dramatically decreased.

It will be understood that the invention disclosed and defined in this specification extends to all alternative combinations of two or more of the individual features mentioned or evident from the text or drawings. All of these different combinations constitute various alternative aspects of the invention.

Eiffel Technologies Limited
By its Registered Patent Attorneys
Freehills Carter Smith Beadle

15 April 2002

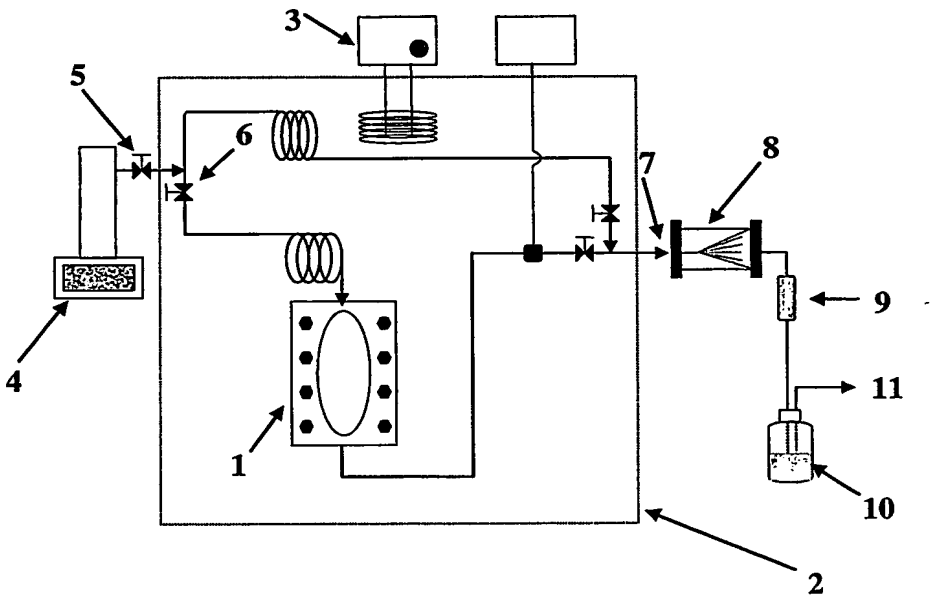


Figure 1

(Prior art)

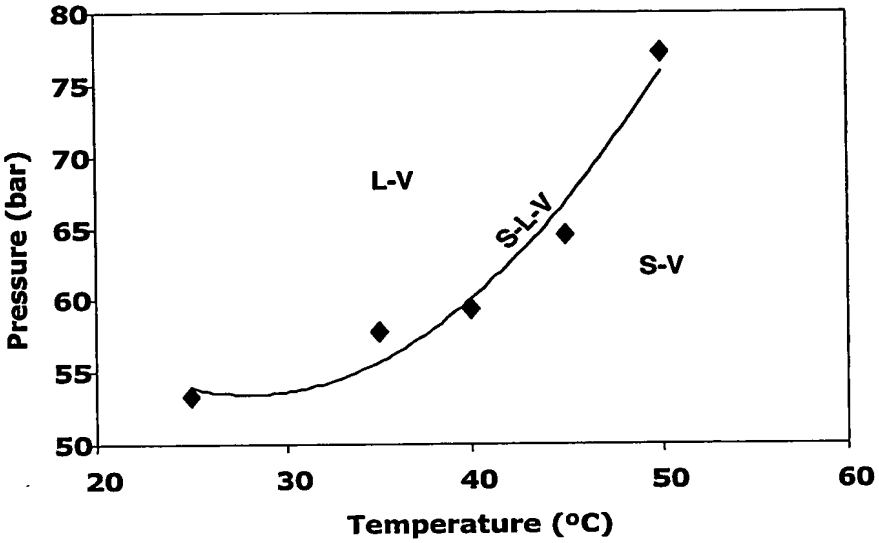

80
75
70
65
60
55
50
Pressure (bar)
L-V
S-L-V
S-V
20
30
40
50
60
Temperature (°C)

Figure 2

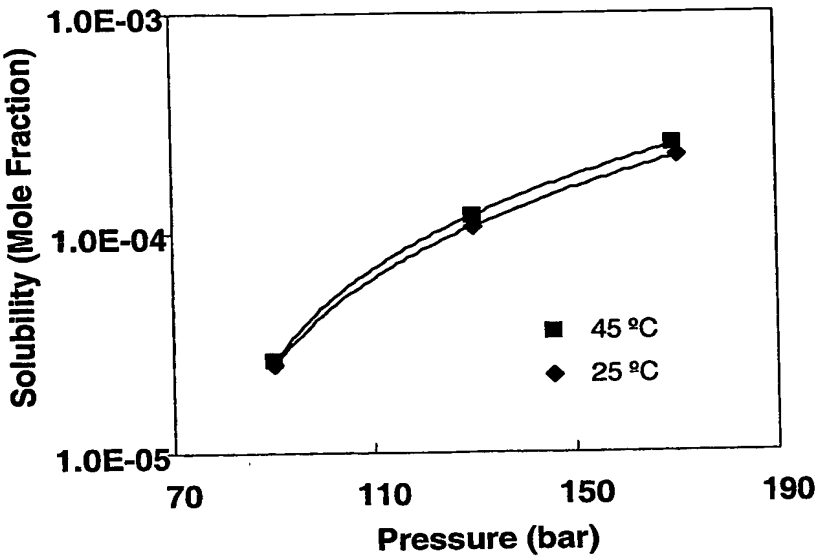
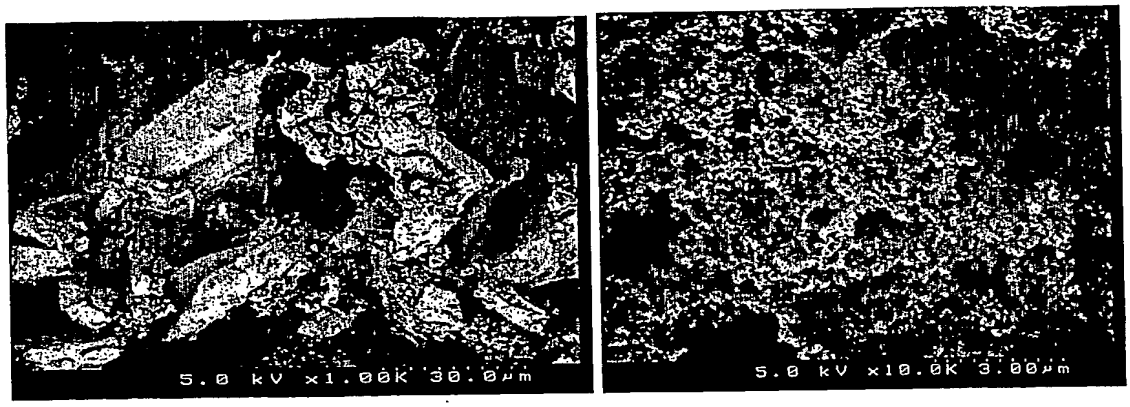

1.0E-03
1.0E-04
1.0E-05
Solubility (Mole Fraction)
70
110
150
190
Pressure (bar)
45 ºC
25 ºC

Figure 3



(a) (b)

Figure 4

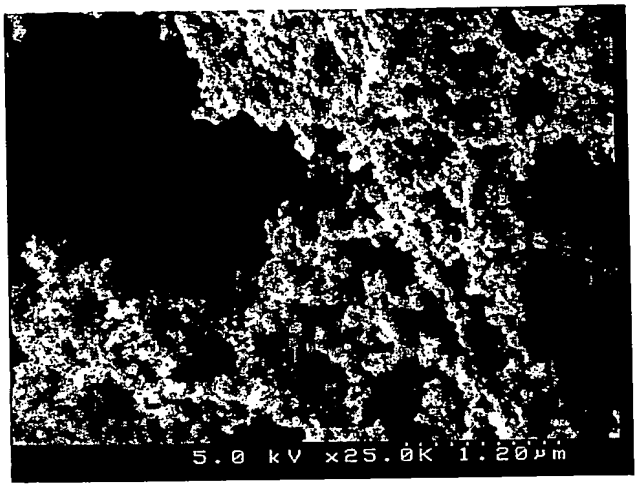


Figure 5

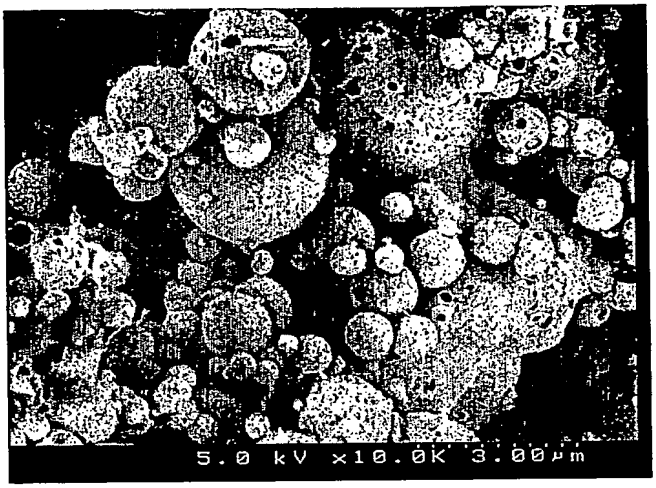
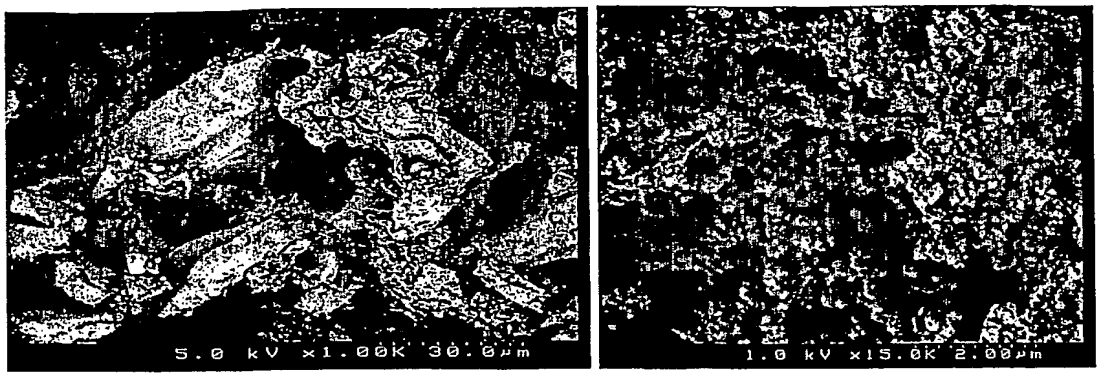


Figure 6



(a) (b)

Figure 7

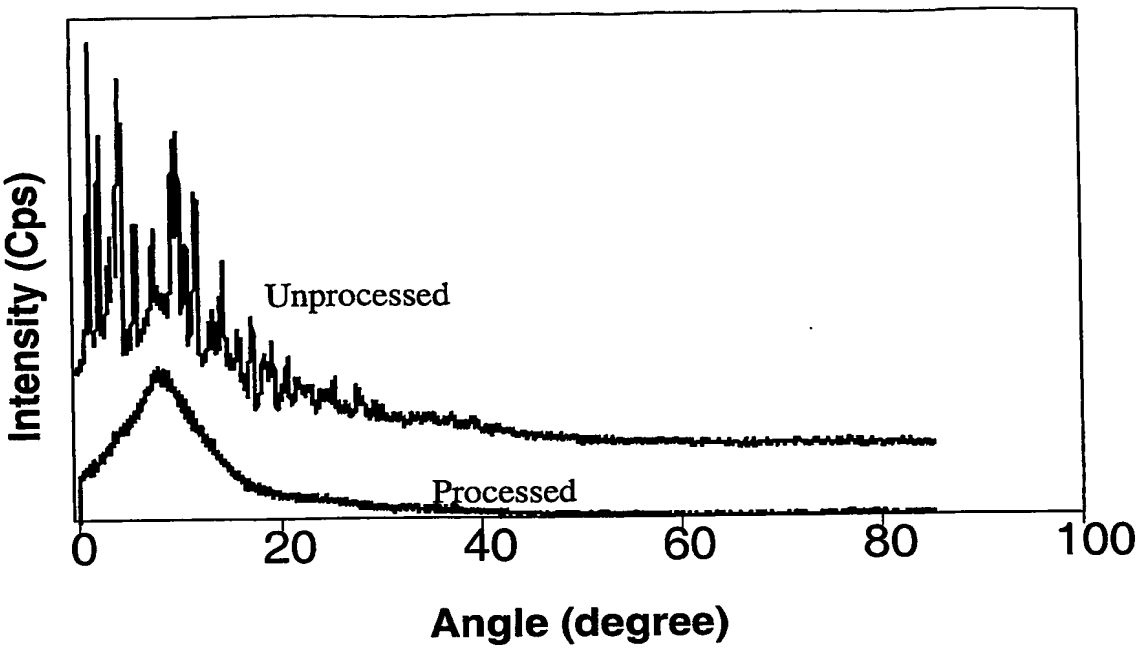

Intensity (Cps)
Unprocessed
Processed
0
20
40
60
80
100
Angle (degree)

Figure 8

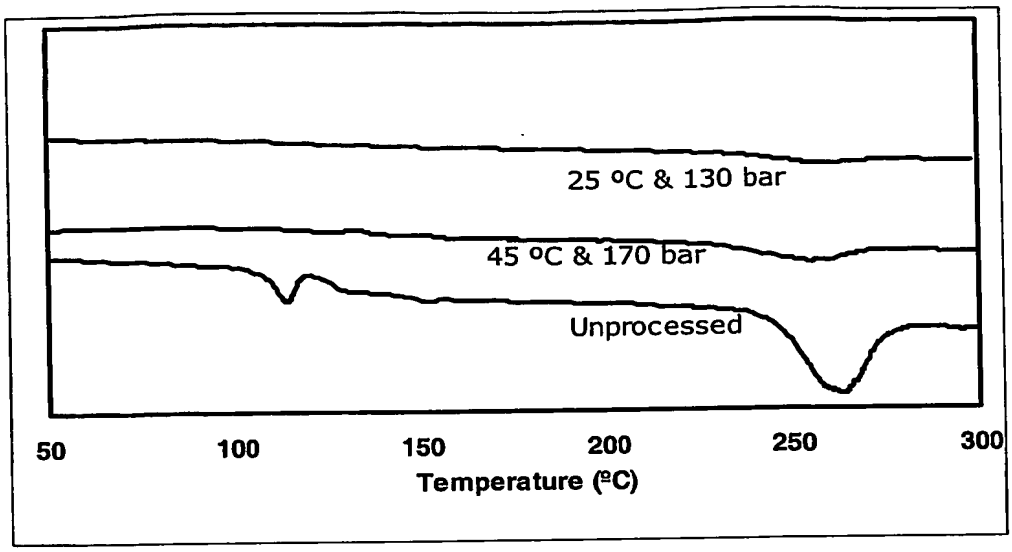
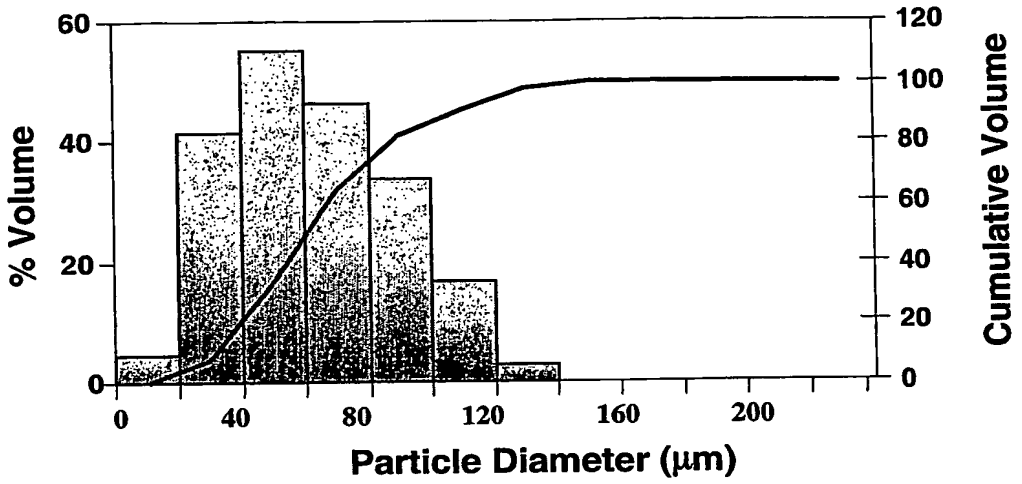

25 °C & 130 bar
45 °C & 170 bar
Unprocessed
50
100
150
200
250
300
Temperature (ºC)

Figure 9

(a)


60
40
20
0
% Volume
120
100
80
60
40
20
0
Cumulative Volume
0
40
80
120
160
200
Particle Diameter (μm)

(b)

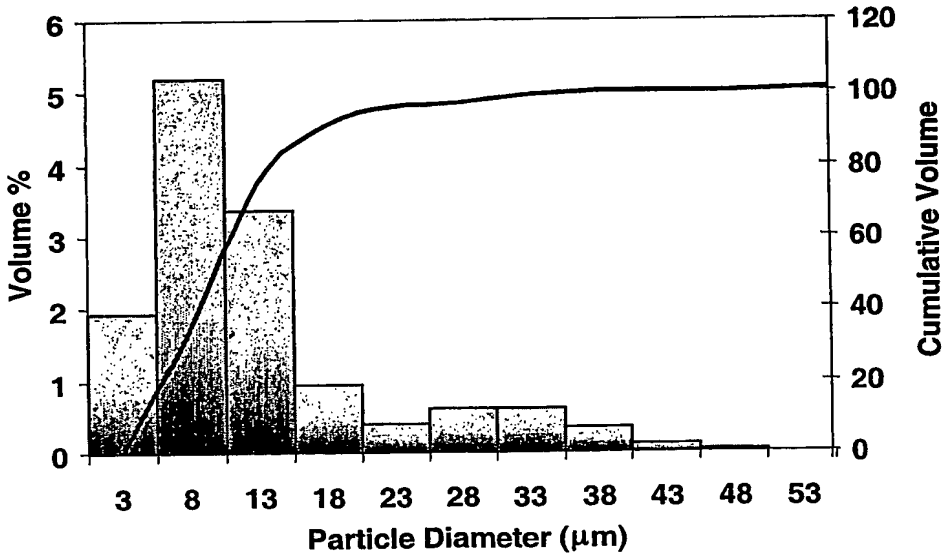

6
5
4
3
2
1
0
Volume %
120
100
80
60
40
20
0
Cumulative Volume
3
8
13
18
23
28
33
38
43
48
53
Particle Diameter (μm)

Figure 10